UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

SEP 15 2010

SUBJECT: Joint review of data for the registration of a new TGAI, NoFly Technical, and a new end use product formulation, NoFly Wettable Powder, containing the fungus *Paecilomyces fumosoroseus* strain FE 9001.

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THROUGH: John Kough, Ph.D., Senior Scientist John 2. Kough
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TO: Kathleen Martin, Regulatory Action Leader
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ACTION REQUESTED: A joint review with PMRA, Canada, of product chemistry, acute toxicity/pathogenicity studies, and waiver requests the registration of a new TGAI, NoFly Technical, and a new end use product formulation, NoFly Wettable Powder, containing the fungus *Paecilomyces fumosoroseus* strain FE 9001submitted by Natural Industries Inc., Houston, TX

CONCLUSION: UPGRADABLE to ACCEPTABLE pending on the response of the registrant to the deficiencies reported in the recommendations.

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

DATA REVIEW RECORD

Active Ingredient:	*Paecilomyces fumosoroseus* strain FE 9001
Product Name:	NoFly Technical, and NoFly Wettable Powder
Company Name:	Natural Industries Inc., Houston, TX
Reg. No:	73314-T
Chemical Number:	115003
Decision Number:	416429
DP Barcode:	381252
MRID No:	47719-01, -02 Product Chemistry & Storage Stability of NOFLY WP 47719-03 – 08 Acute Toxicity & Sensitization Studies of NOFLY WP 477920-01 Product Chemistry of NOFLY Technical 477920-02 – 07 Acute Toxicity & Waiver requests of NOFLY Technical

BACKGROUND:

Paecilomyces fumosoroseus is a naturally-occurring fungus in most countries of the world and occurs in various soil types at very low densities. The fungus has frequently been isolated from lepidopteran cadavers in Europe. Isolates occur in the Netherlands, Ireland, and Spain. The fungus has also been found in various non-European countries such as China, Japan, Ghana, Pakistan, India, and the USA. Target Organisms: NoFly WP is intended to control all stages of whitefly (eggs, N1, N2, N3 and N4 larvae, and adults). The ability to infect whitefly eggs is unique among entomopathogenic fungi. The target organisms are the whiteflies Trialeurodes vaporariorum, Bemisisa tabaci, Bemisia argentifolii, and Lecanoideus floccissimus. In its natural environment, *P. fumosoroseus* strain FE 9901 is dispersed as conidia or blastospores, either by air or water movement or by other insects and/or mites. The mode of infection of the various entomopathogenic fungi is basically similar where a typical infection cycle proceeds as such: conidial attachment, germination, penetration, vegetative growth, and then conidiogenesis. The infection cycle of *P. fumosoroseus* strain FE 9901 is particularly rapid under optimal conditions. Initially, conidia are attached to the cuticle or the dorsum of immature or adult whiteflies. First symptoms of infection typically are apparent within one to two days after contact. Conidia and hyphae are present in the host hemocoel within one day. Fungal multiplication takes place through formation of hyphal bodies or blastospores. The mycelium is present on the dorsum of the insect body within two days. Sporulation occurs within three days and reaches a maximum within five to seven days. Conidiospores generated from the mycelial growth on dead insects can infect additional live target pests. The ability of *P. fumosoroseus* strain FE 9901 to produce infections on eggs of two novel whitefly species was evaluated under laboratory conditions. The strain produced infections on the N1 and N4 nymph stages and significantly reduced the hatching capacity of eggs of *Lecanoides floccissimus*. It also produced infections on *Aleurodicus disperse* N1 and N4 nymph stages and reduced the percent of hatching of eggs by 50%.

DISCUSSION:

With the notable exception of beauverolides, no impurities of toxicological significance are associated with the TGAI or EP. NoFly Technical contains [redacted] Test substances in all toxicity tests included these components in addition to the MPCA. Therefore, the potential toxicity of inert materials associated with the active ingredient in both TGAI and EP has been evaluated. [redacted] Therefore, no side-products from chemical reactions are likely. The acute pulmonary infectivity and toxicity study was done in ferrets and not in rats. Although we usually prefer that this study be done in rats, the applicant has given enough justification for the use of ferrets that is acceptable. Also, in the acute intraperitoneal Infectivity study in rats, the applicant has studied microbial clearance only in blood samples and has not studied microbial clearance in other body organs.

RECOMMENDATION: The following data are required for the unconditional registration of NoFly Technical, and NoFly Wettable Powder:

1- A five batch analysis of EP and TGAI from the proposed manufacturing site must be submitted.

2- Microbial clearance from body organs in the acute intraperitoneal infectivity study in rats.

SUMMARY OF DATA SUBMITTED:

Product Chemistry & Storage Stability (MRID 47719-01, 47719-02, & 47720-01)

NoFly Technical is a wettable powder consisting of [redacted]. NoFly WP is a wettable powder consisting of [redacted]. NoFly Technical is a slightly cohesive, dusty powder with a bulk density of 0.6 — 0.7 g/cc and 99% of the particles are less than 100µm. Its color is lilac and has a yeast-like odor. A 1% suspension of NoFly Technical in water has a pH of 6.5 — 7.5. NoFly WP is a powder with a bulk density of 0.65 — 0.75 g/cc. Its color

is lilac (Pantone 435M/662C) and has a yeast-like odor. A suspension of NoFly WP in water has a pH of 6.5 — 7.5. The potency estimation data for NoFly Technical supports a nominal guarantee of 8.1×10^9 CFU/g. A lower limit of [redacted] for the TGAI is also supported by the data. The potency estimation data for NoFly WP supports a nominal guarantee of 2.0×10^9 CFU/g. A lower limit of [redacted] for the EP is also supported by the data.

Manufacturing Process:

The TGAI, NoFly Technical, and the formulated EP, NoFly WP, are produced [redacted] process of *P. fumosoroseus* strain FE 9901. Production of NoFly Technical involves typical procedures for growth of microbial products and is summarized in Figure 1

Figure 1. Flowchart for Manufacture of *NoFly™ Technical*

[redacted]

Formation of Unintentional Ingredients:

NoFly Technical

Parameter Measured	Batch Number					Mean	Quality Control Specification
	Pf 0702	Pf 0706	Pf 0708	Pf 0712	Pf 0715		
Coliform count	Abs	Abs	Abs	Abs	Abs	Abs	Absent in 0.1g
Gram (-) Enteric bacteria	Abs	Abs	Abs	Abs	Abs	Abs	Absent in 0.1g
Salmonella/Shigella	Abs	Abs	Abs	Abs	Abs	Abs	Absent in 0.1g

Lactobacilli	0	0	0	0	0	0	$< 10^3$ CFU/g
Other fungi	0	0	0	0	0	0	< 1% of P. fumosoroseus

NoFly WP

Parameter Measured	Batch Number					Mean	Quality Control Specification
	FE07 0920	FE07 0927	FE07 1010	FE07 0207	FE08 0409		
Coliform count	Abs	Abs	Abs	Abs	Abs	Abs	Absent in 0.1g
Gram (-) Enteric bacteria	Abs	Abs	Abs	Abs	Abs	Abs	Absent in 0.1g
Salmonella/Shigella	Abs	Abs	Abs	Abs	Abs	Abs	Absent in 0.1g
Lactobacilli	0	0	0	0	0	0	$< 10^3$ CFU/g
Other fungi	0	0	0	0	0	0	< 1% of P. fumosoroseus

Analysis for Other Unintentional Ingredients demonstrated that known mammalian toxins are not produced or are not present in final products.

Storage Stability Testing

A storage stability study was conducted on FuturEco NoFly WP formulation; batch number FE 051216 before and after storage for 6 months at 4°C. The viable count test (CFU/g) of the formulation showed a 10-fold reduction over the storage period of six months set at 4°C (± 2 °C). There was no significant difference in the physical test results before and after storage.
The reviewers agree that the details of the study support the conclusions drawn above. However, since the one batch of EP that was tested was not produced at the proposed manufacturing site, differs in formulation and the relative viability of EP after six months was mediocre, the proposed storage statement of six months at 4°C cannot be supported. A storage statement for a period of 3 months at 4°C can be supported at this time with the condition that new data be produced using the proposed formulation, manufactured at the proposed site.

Deficiencies: A storage stability study must be conducted using the proposed formulation of NoFly WP from the proposed manufacturing site.

Acute Oral Toxicity – Rat **MRID # 47719-03**
In an acute oral toxicity study, Futureco NoFly (containing 2×10^9 colony forming units of *Paecilomyces fumosoroseus* strain FE 9901 per gram) was administered to three female Sprague-Dawley rats as a gavage dose of 5000 mg/kg bw following the up and down procedure. The study

was terminated following the stopping rules for this procedure. Each animal was then observed for a period of up to 14 days. Oral LD50 is > 5000 mg/kg bw.
None of the animals died or showed any symptoms of intoxication following treatment. The test material had no negative effect upon the body weight and no gross pathological changes were noted. Based on the results of this study, Futureco NoFly is of LOW Toxicity (EPA Toxicity Category IV) to the rat via the oral route. This acute oral study is classified acceptable. This study satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 425) in the rat.
CLASSIFICATION: **ACCEPTABLE**

Acute Dermal Toxicity – Rat **MRID # 47719-04**
In an acute dermal toxicity study, a group of 7 week-old Sprague-Dawley rats (5/sex) was dermally exposed to Futureco NoFly (containing 2×10^9 colony forming units of *Paecilomyces fumosoroseus* strain FE 9901 per gram) at a limit dose of 5050 mg/kg bodyweight for 24 hours to an area of not less than 10% of the total body surface. Following exposure, the animals were observed for a period of 14 days.

Dermal LD_{50}	Males	> 5050 mg/kg bw
	Females	> 5050 mg/kg bw
	Combined	> 5050 mg/kg bw

There were no mortalities and all the test animals appeared normal for the duration of the study. All animals gained weight with the exception of one female which failed to gain weight between Days 7 and 14. At necropsy, two male rats had pale lungs at study termination.
Based on these results, Futureco NoFly is of LOW Toxicity to the rat via the dermal route and is minimally irritating (i.e., mean irritation score=0.1) following a 24-hour exposure. This acute dermal toxicity study is classified as acceptable. This study satisfies the guideline requirement for an acute dermal toxicity study (OPPTS 870.1200: OECD 402) in the rat.
CLASSIFICATION: **ACCEPTABLE,** Toxicity Category IV

Acute Inhalation Toxicity- Rat **MRID # 47719-05**
In an acute inhalation toxicity study, a group of 10-week old Sprague-Dawley rats (5/sex) were exposed by the inhalation route to undiluted Futureco NoFly (containing 2×10^9 colony forming units of *Paecilomyces fumosoroseus* strain FE 9901 per gram) for 4 hours to nose only at a concentration of 2.18 mg/L. Animals then were observed for 14 days.

Inhalation LC50	Males	> 2.18 mg/L
	Females	> 2.18 mg/L
	Combined	> 2.18 mg/L
	Limit test	

There were no mortalities noted throughout the study. Clinical observations included piloerection and decreases in activity which resolved by Day 3. All test animals gained body weight throughout the study period and no observable abnormalities were observed at gross necropsy.
Based on the results of this study, Futureco NoFly is of LOW Toxicity in male and female Sprague-Dawley rats via the inhalation route. This acute inhalation study is classified as acceptable. It also satisfies the guideline requirement for an acute inhalation study (OPPTS 870.1300; OECD 403) in the rat.

CLASSIFICATION: **ACCEPTABLE,** Toxicity Category IV

Primary Eye Irritation – Rabbit **MRID # 47719-06**

In a primary eye irritation study, 0.1 g of Futureco NoFly WP (containing 88% *Paecilomyces fumosoroseus* strain FE 9901) was instilled into the conjunctival sac of the left eye of 3 adult male New Zealand white rabbits. The treated eyes were not washed following application. The untreated right eye of each test animal served as a negative control. Test animals then were observed for 72 hours. Irritation was scored by the method of Draize.

After 1 hour, some redness (grade 1) was observed in one rabbit, and slight to moderate increases in excretion discharge were noted in 3 rabbits. All test animals were free of irritation at the 24-, 48- and 72-hour observation time points. In this study, Futureco NoFly WP (*P. fumosoroseus* strain FE 9901) is SLIGHTLY IRRITATING to the eyes of New Zealand white rabbits.

This study is classified as acceptable. This study satisfies the guideline requirement for a primary eye irritation study (OPPTS 870.2400; OECD 405) in the rabbit.

CLASSIFICATION: **ACCEPTABLE,** Toxicity Category IV

Dermal Irritation – Rabbit **MRID # 47719-07**

In a primary dermal irritation study, adult male New Zealand white rabbits (3) were dermally exposed to 0.5 g Futureco NoFly WP (containing 88% *Paecilomyces fumosoroseus* strain FE 9901 [ARSEF]) in water for 4 hours to an area of skin of approximately 6 cm2. Animals then were observed for 72 hours. Irritation was scored by the method of Draize.

Very slight erythema was noted in two test animals 1 hour after patch removal. All test animals were free of irritation at the 24-, 48- and 72-hour observation time points. Based on the calculated maximum irritation score and an irritation index of 0.7/8 (1 h) and 0.17 (respectively), Futureco NoFly WP is slightly irritating to the skin of male New Zealand white rabbits.

This study is classified as acceptable. This study satisfies the guideline requirement for a primary dermal irritation study (OPPTS 870.2500; OECD 404) in the rabbit.

CLASSIFICATION: **ACCEPTABLE,** Toxicity Category IV

Dermal Sensitization – Guinea Pig **MRID # 47719-08**

In a skin sensitization study with Futureco NoFly WP (containing 88% *Paecilomyces fumosoroseus* strain FE9901), Dunkin Hartley guinea pigs (10 females) were tested using Magnusson and Kligman method. Guinea pigs were subjected to a two-stage induction procedure; a 1% intradermal injection and a 25% topical application. Two weeks following the last induction exposure, a challenge dose was applied as a 25% topical application. Five additional female guinea pigs were exposed to the vehicle during induction then challenged with the test material. This group served as a negative control. The dermal reactions observed in test animals (left flank) following challenge included confluent, intense erythema and swelling. No visible dermal reactions were observed to the vehicles (right flank) or control animals. In this study, Futureco NoFly WP was a sensitizer in guinea pigs following the Magnusson and Kilgman procedure. This study satisfies the guideline requirement for a dermal sensitization study (OPPTS 870.2600; OECD 406) in the guinea pig.

CLASSIFICATION: **ACCEPTABLE,** Toxicity Category IV

Acute Oral Infectivity and Toxicity – Rat **MRID # 47720-02**

In an acute oral toxicity study, groups of fasted, young adult (6–7 weeks) Wistar rats (15 rats/sex)

were given a single oral dose of the MPCA, *Paecilomyces fumosoroseus* strain FE 9901 (100%; 1.0×10^9 CFU/g), in 0.05% Tween 80 at a single dose of 10^8 CFU per animal. The animals were then observed for a period of up to 21 days with scheduled sacrifices 24 hours after application and on Days 3, 7, 14 and 21. Another group of rats (4 rats/sex) were untreated and served as a negative control.

Oral LD50	Males	$> 10^8$ CFU/animal
	Females	$> 10^8$ CFU/animal
	Combined	$> 10^8$ CFU/animal

None of the animals died and no clinical observations were noted during the study period. No gross pathological changes were noted in sacrificed test animals. Based on the results of this study, *P. fumosoroseus* strain FE 9901 is of LOW Toxicity and is not infective or pathogenic in the rat.

CLASSIFICATION: **ACCEPTABLE,** Toxicity Category IV

Acute Pulmonary Infectivity and Toxicity – Ferret **MRID # 47720-04**

In an acute pulmonary infectivity and toxicity study, groups of young adult ferrets (14/sex) were exposed by the intranasal route to the microbial pest control agent (MPCA), *Paecilomyces fumosoroseus* strain FE 9901 (100%; 1.0×10^9 CFU/g), in 0.05% Tween 80 at a nominal dose of 1×10^8 CFU (in 0.5 mL) per animal. Animals were then observed for up to 21 days. Another group of ferrets (4 ferrets/sex) were untreated and served as a negative control.

Pulmonary LD50	Males	$> 0.9\times10^8$ CFU/animal
	Females	$> 0.9\times10^8$ CFU/animal
	Combined	$> 0.9\times10^8$ CFU/animal

None of the animals died or showed any symptoms of intoxication following treatment. Some animals lost weight during the first week of the study and it is not known if these effects were reversed by the end of the study since these data were not provided in the study report. No gross pathological changes were noted. Microbiological examination of lungs, other prescribed organs and body fluids proved minimal persistence and no infectivity of MPCA.

Based on the results of this study, *P. fumosoroseus* strain FE 9901 is of SLIGHT Toxicity and the MPCA is not infective or pathogenic in the ferret. This acute pulmonary infectivity and toxicity study is classified as ACCEPTABLE.

Ferrets are usually not used for acute pulmonary toxicity/pathogenicity testing, but is acceptable because of the justification given for their use

CLASSIFICATION: **ACCEPTABLE,** Toxicity Category IV

Acute Intraperitoneal Infectivity **MRID # 47720-05**

In an acute intraperitoneal infectivity study, a group of young adult Wistar rats (3/sex) were injected with the MPCA, *Paecilomyces fumosoroseus* strain FE 9901 (100%; 1.0×10^9 CFU/g), in 0.05% Tween 80 at a dose of 10^7 CFU per animal. Animals were then observed for up to 21 days. Another group of rats (4 rats/sex) were untreated and served as a negative control. Based on the results of this study, *P. fumosoroseus* strain FE 9901 is not infective or pathogenic in the rat via the intraperitoneal route of exposure. None of the animals died or showed any symptoms of intoxication after treatment. The test item had no negative effect upon the body weight and no gross pathological changes were noted. Microbial clearance was shown from blood samples but not from body organs. This intraperitoneal infectivity study is classified as supplemental.

CLASSIFICATION: **SUPPLEMENTAL**

Hypersensitivity Incidence **MRID # 47720-06**

The registrant submitted a statement indicating that there were no reports of hypersensitivity reactions with the microbial pest control agent (MPCA), *Paecilomyces fumosoroseus* strain FE 9901, among researchers, product developers and manufacturers. An end-use product containing *P. fumosoroseus* strain FE 9901 was determined to be a sensitizer in a skin sensitization study and 21 reports of hypersensitivity reactions were found in the PubMed database using the keywords "*paecilomyces*" and "allergy". Based on the assumption that most microorganisms contain substances that would elicit allergic reactions, the technical grade of the active ingredient, NoFly Technical, is considered a potential sensitizing agent.

CLASSIFICATION: **ACCEPTABLE**